Pharmaceutical biotechnology Lecture-4

Excipients for parenteral formulations of biotech product

Buffer components

Preservatives, Anti-oxidants and osmotic agents

Buffer components

- Buffer selection is an important part of the formulation process, because of the pH dependence of protein solubility and physical and chemical stability.
- Buffer systems regularly encountered in biotech formulations are

1. phosphate
2. citrate and
3. acetate

- A good example of importance of the isoelectric point (its negative logarithm is equal to pI) is the solubility profile of human growth hormone (hGH, pI around 5) as presented in Figure 4.4.

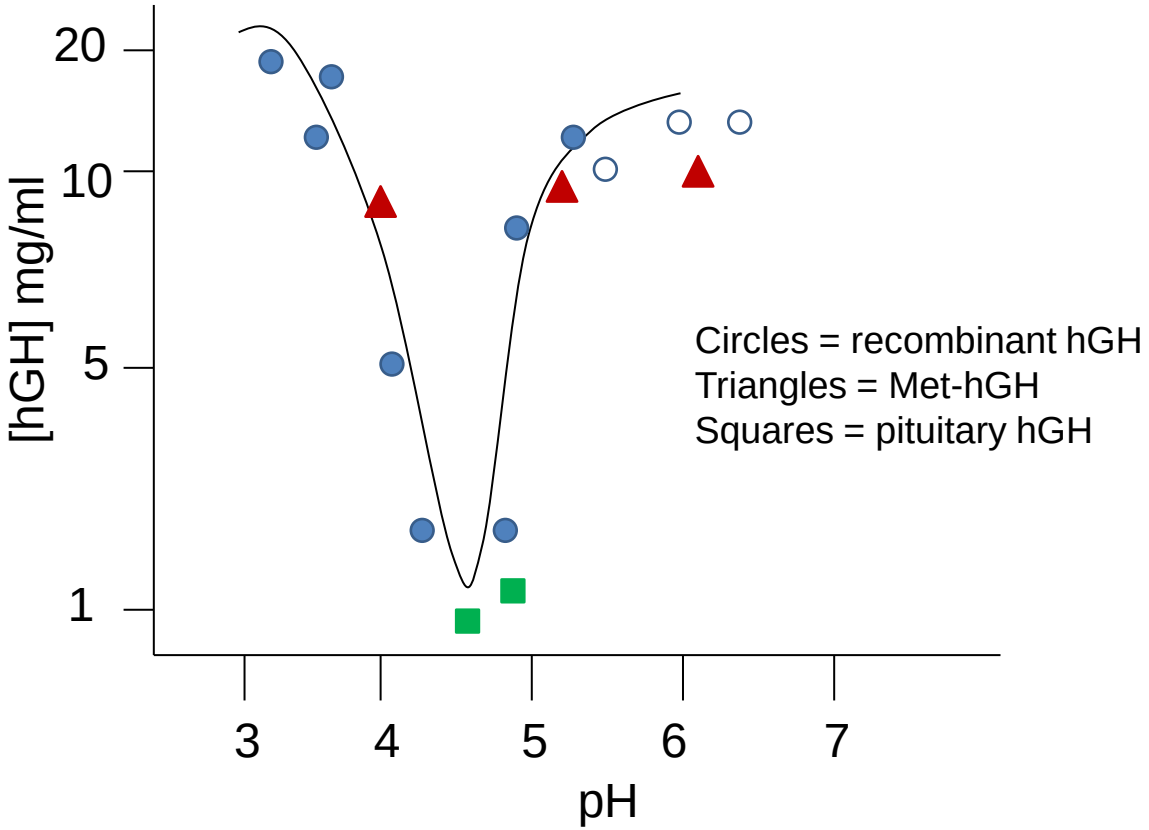


Figure 4.4. A plot of the solubility of various forms of hGH as a function of pH. The closed symbols mean that precipitate was present in the dialysis tube after equilibration, whereas open symbols mean that no solid material was present, and thus the solubility is at least this amount.

The isoelectric point (pI)

- pH of a solution at which the net primary charge of a protein becomes zero.
- At a solution pH that is above the pI the surface of the protein is predominantly negatively charged and like-charged molecules will exhibit repulsive forces.
- At a solution pH that is below the pI, the surface of the protein is predominantly positively charged and repulsion between proteins occurs.
- At the pI the negative and positive charges cancel, repulsive electrostatic forces are reduced and the attraction forces predominate.
- The attraction forces will cause aggregation and precipitation.
- The pI of most proteins is in the pH range of 4-6.

- pI: is the pH at a particular molecule carries no net electrical charges (overall charge).
- Thus molecule is affected by pH of its surrounding environment and can become more positively or negatively charged due to the gain or loss, respectively, of (H^+).
- Such molecules have minimum solubility in water or salt solutions at the pH that corresponds to their **pI** and often precipitate out of solution.

Even short, temporary pH changes can cause aggregation. Explain why?

- These conditions can occur, for example, during the freeze-drying process, when one of the buffer components is crystallizing and the other is not.
- In a phosphate buffer, Na_2HPO_4 crystallizes faster than NaH_2PO_4.
- This causes a pronounced drop in pH during the freezing step.
- Other buffer components do not crystallize, but form amorphous systems and then pH changes are minimized.

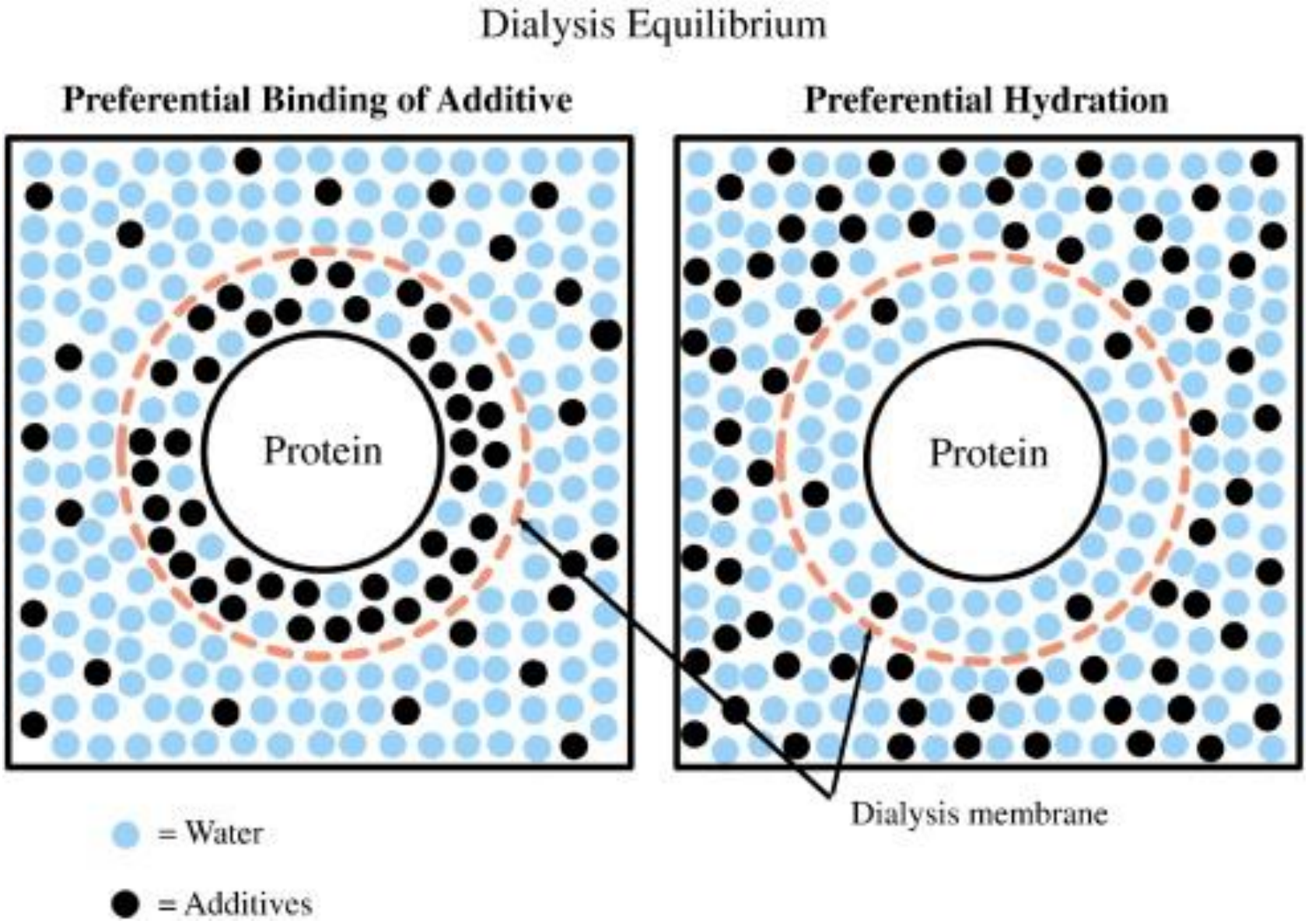
Preservatives and Anti-oxidants

- Methionine, cysteine, tryptophane, tyrosine and histidine are amino acids that are readily oxidized.
- Proteins rich in these amino acids are susceptible to oxidative degradation.
- The replacement of oxygen by **inert gases** in the vials helps to reduce oxidative stress.
- More-over, the addition of anti-oxidant such as **ascorbic acid or sodium formaldehyde sulfoxylate** can be considered.
- Interestingly, destabilizing effects on proteins have been described for anti-oxidants, as well; e.g. ascorbic acid can act as an oxidant in the presence of a number of heavy metals.

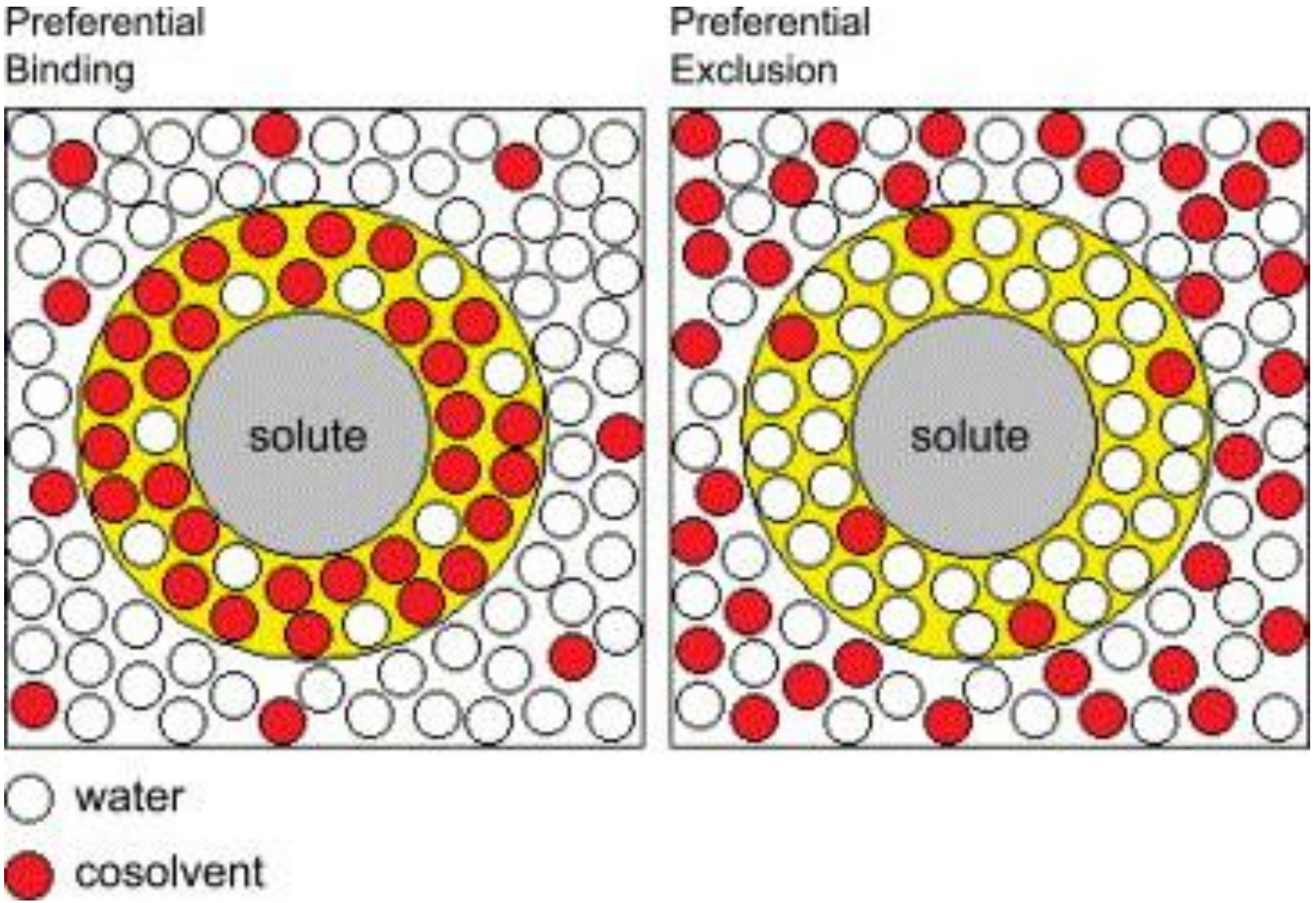
- Certain proteins are formulated in the container designed for multiple injection schemes.
- After administering the first dose, contamination with microorganism may occur and the preservatives are needed to minimize growth.
- Usually, these preservatives are present in concentrations that are bacteriostatic rather than bactericide in nature.
- Antimicrobial agents mentioned in the USP XXIV are the mercury-containing pheylmercuric nitrate and thimerosal and p-hydroxybenzoic acids, phenol, benzyl alcohol and chlorobutanol.

Osmotic Agents

- For proteins, the regular rules apply for adjusting the tonicity-of parenteral products.
- Saline and mono- or disaccharide solutions are commonly used.
- These excipients may not be inert; they may influence protein structural stability. For example, sugars and polyhydric alcohol can stabilize the protein structure through the principle of preferential exclusion.
- These additives enhance the interaction of the solvent (water structure promoters) with the protein and are themselves excluded from the protein surface layer; the protein is preferentially hydrated.



Schematic representation of preferential binding and preferential hydration. Adapted from Timasheff (1992a).


Preferential
Binding
Preferential
Exclusion
solute
solute
water
cosolvent

- This phenomenon can be monitored through an increased thermal stability of the protein.
- Unfortunately, a strong “preferential exclusion” effect enhances the tendency of proteins to self-associate.

Shelf Life of Protein Based Pharmaceuticals

- Protein can be stored
- (1) as an aqueous solution
- (2) in freeze-dried form, and
- (3) in dried form in a compacted state (tablet)

The stability of protein solutions strongly depends on factors such as pH, ionic strength, temperature, and the presence of stabilizers.

For example, the Figure 4.5 shows the pH dependence of α_1-antitrypsin and clearly demonstrates the critical importance of pH on the shelf-life of proteins.

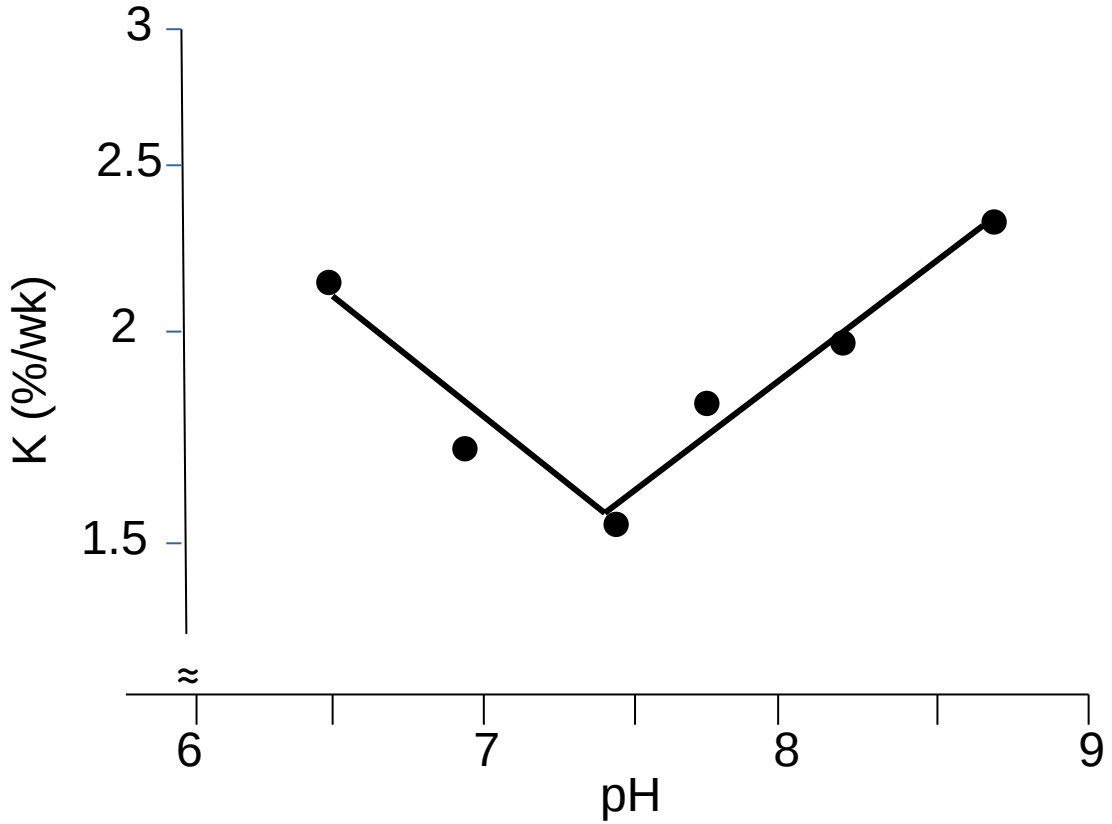
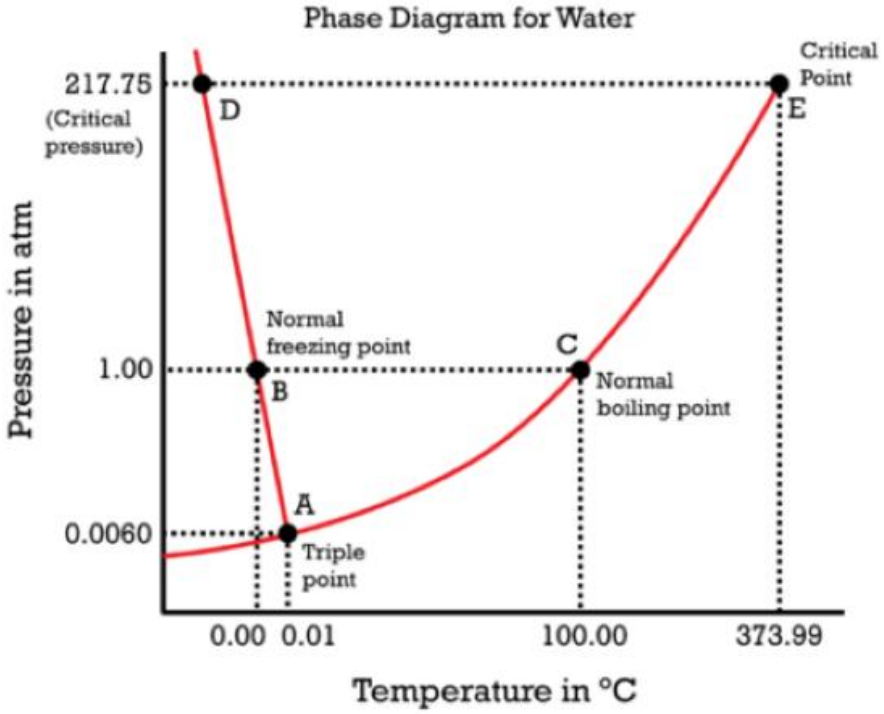


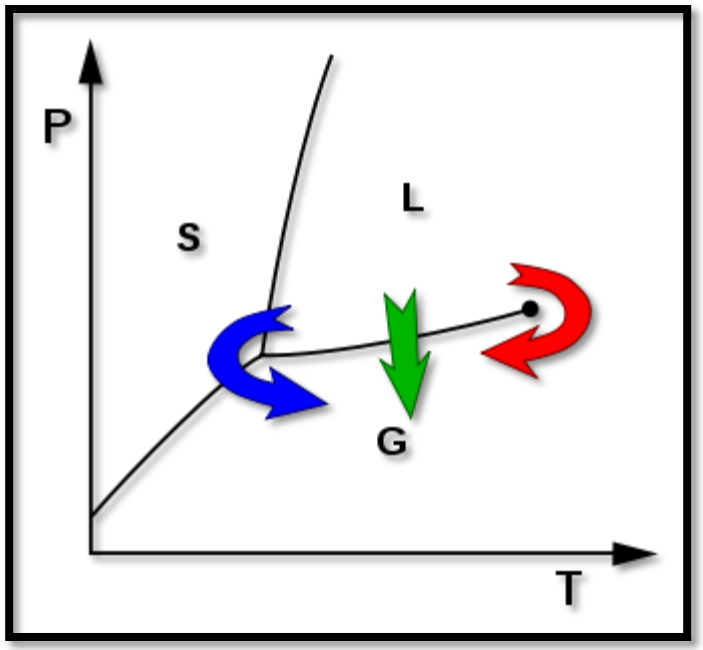
Figure 4.5. pH stability profile (at 25°C) of monomeric recombinant α_1-antitrypsin (rAAT) by size exclusion-HPLC assay. K = degradation rate constant. Monomeric rAAT decreased rapidly in concentration both under acidic and basic conditions. Optimal stability occurred at pH 7.5.

Freeze-Drying of Proteins

- Proteins in solution often do not meet the preferred stability requirements for industrially pharmaceutical products (>2 years), even when kept permanently under refrigerator conditions (cold chain).
- The abundant presence of water promotes chemical and physical degradation processes.

Sublimation: conversion from solid to gas without passing through liquid state





In a typical phase diagram, the boundary between gas and liquid runs from the triple point to the critical point. Freeze-drying (blue arrow) brings the system around the triple point, avoiding the direct liquid-gas transition seen in ordinary drying time (green arrow).

Importance of Freeze Drying

- Freeze-drying may provide the desired stability. During freeze-drying water is removed via sublimation and not by evaporation.
- Three stages can be discerned in the freeze-drying process:

  (1) a freezing step

  (2) the primary drying step, and

  (3) the secondary drying step.

  Table 4.2 explains what happens during these stages.

Table 4.2. Three stages in the freeze drying process of protein formulations.

1. ***Freezing***

- The temperature of the product is reduced from ambient temperature to a temperature below the eutectic temperature (Te), or below the glass transition temperature (Tg) of the system. A Tg is encountered if amorphous phases are present.

2. ***Primary drying***

- Crystallized and water not bound to protein/excipients is removed by sublimation. The temperature is below the Te or Tg; the temperature is for example -40^{o}C and reduced pressures are used.

3. ***Secondary drying***

- Removal of water interacting with the protein and excipients. The temperature in the chamber is kept below Tg and rises gradually, e.g., from -40^{o}C to 20^{o}C.

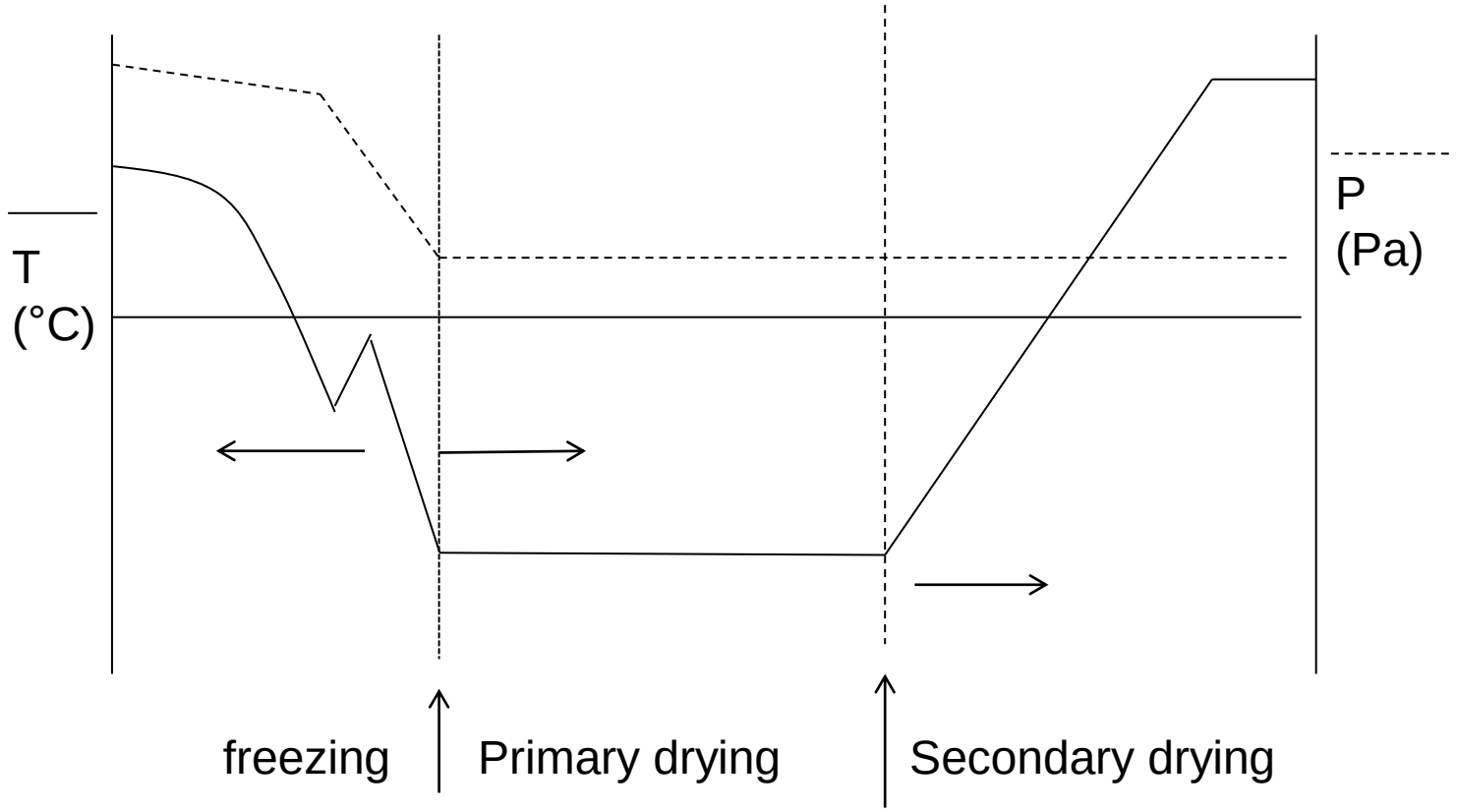


Figure 6 Example of freeze-drying protocol for systems with crystallizing water. Abbreviations: T, temperature; P, pressure.

Freeze Drying

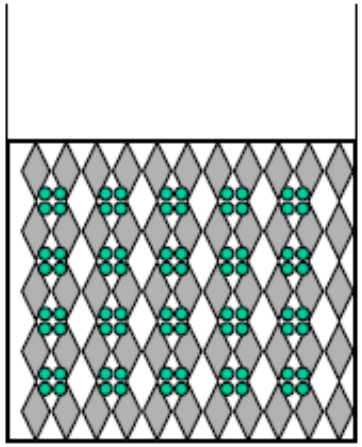
Solution

Temperature
Time
Pressure

Powder

Freezing step

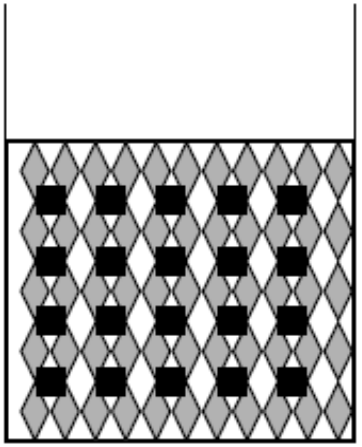
Crystalline Solutes



After Freezing
(Freeze Concentrate)

Some solutes crystallize with ice during freezing

- Crystalline solutes



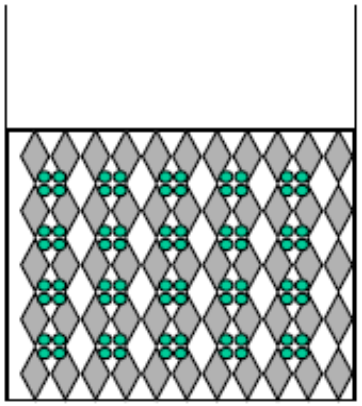
Eutectic Mixture

The temperature where solute and ice both exist in a rigid crystalline state is the "eutectic temperature".

For example, NaCl forms a eutectic mixture containing 23.3%NaCl and melts at -21.13°C.

Freezing step

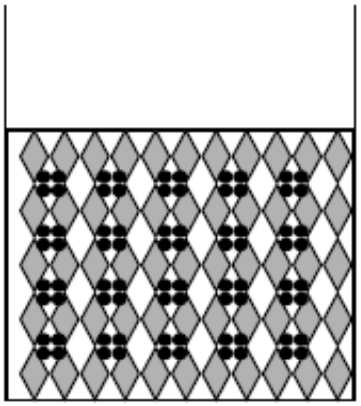
Amorphous Solutes



After Freezing
(Freeze Concentrate)

Most solutes don't crystallize and form a random (amorphous) viscous glassy phase

- Amorphous solute



Glassy Mixture

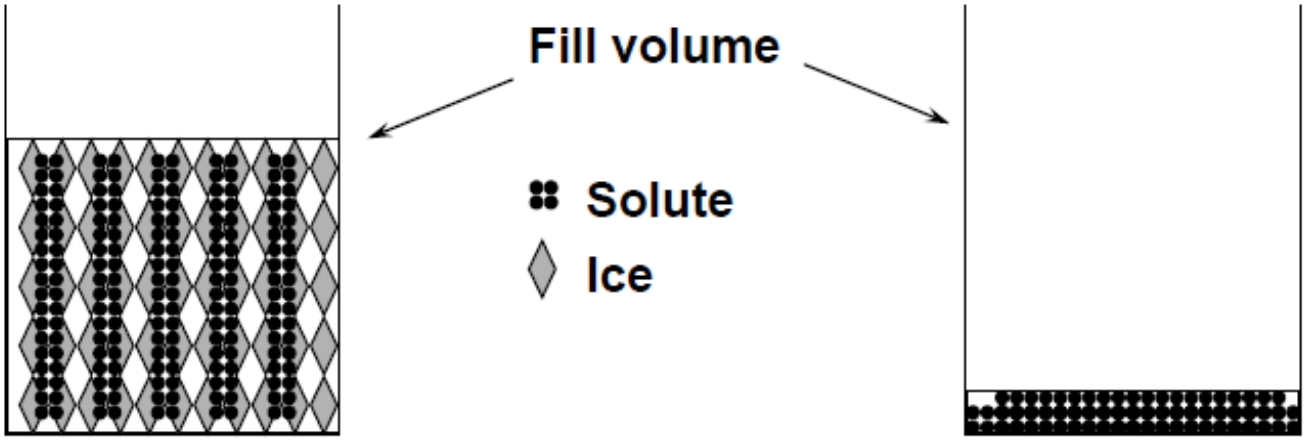
In these systems the viscosity of solute phase increases until the solute is completely immobile and behaves like a glass.

The temperature where the solute behavior changes from solution to a rigid glass is the "glass transition" temperature.

- The freeze-drying of a protein solution without the proper excipients causes, as a rule, irreversible damage to the protein.
- Table 4.3 lists excipients typically encountered in successfully freeze-drying protein products.

Collapse lead to loss of material structure

Product Collapse - during freeze drying product temperature exceeds the collapse temperature and the material "collapse" as ice is sublimed.



- After ice sublimed a dried residue of solute is produced.

Table 4.3. typical excipients in a freeze-dried protein formulation

1. Bulking agents: mannitol/ glycine

- Reason: elegance/ blowout prevention
  - Blowout is the loss of material taken away by the water vapor that leaves the vial.
  - It occurs when little solid material is present in the vial.

2. Collapse temperature modifier: dextran, albumin/ gelatine

- Reason: increase collapse temperature

3. Lyoprotectant: sugars, albumin

- Reason: protection of the physical structure of the protein.
  - Mechanism of action of lyoprotectants is not fully understood. Factors that might play a role are:

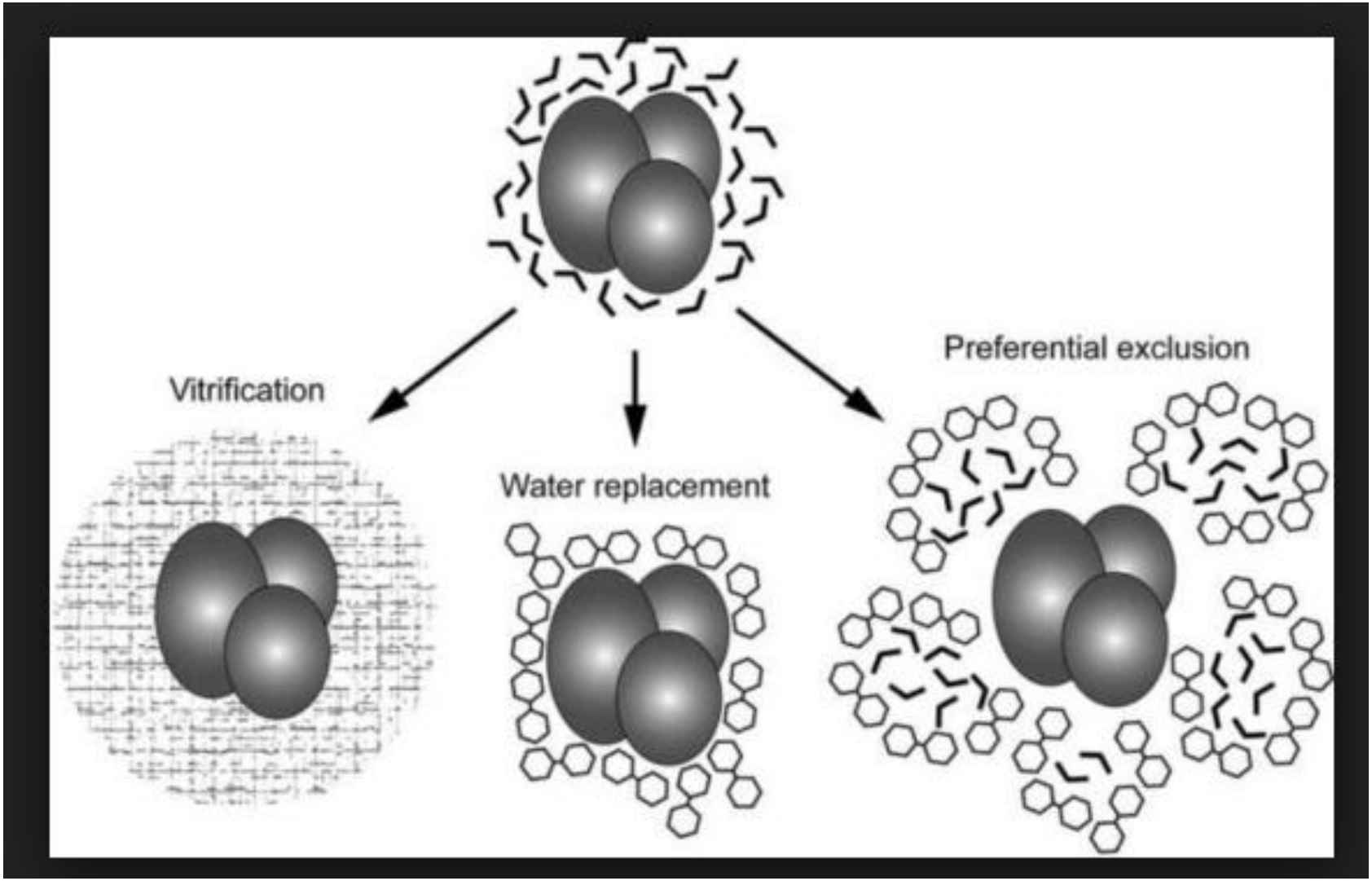
Mechanisms of action of lyoprotectants

1. Lyoprotectants replace water as stabilizing agent (water replacement theory),
2. Lyoprotectants increase the Tg of the cake/ frozen system
3. Lyoprotectants will absorb moisture from the stoppers
4. Lyoprotectants slow down the secondary drying process and minimize the chances for overdrying of the protein.

- Overdrying might occur when residual water levels after secondary drying become too low.

Lyoprotectants

- Substance which protect drugs espacially proteins from degradation during drying (dehydration).
- **Two mechanisms**
  - **Water replacement theory:** a good stabilizer serves as a water substitutes by hydrogen-bonding to the dried protein.
  - **Vitrification theory:** the protein and stabilizer are both amorphous glasses immobilized together where the stabilizer protect the protein from degradation.
- Example:
  - Sugars: Mannitol, Lactose, Maltose, Maltodextrin, Trehalose, Sucrose
  - Amino Acids: Glycin, histadine, arginine



https://www.researchgate.net/publication/51489026_The_use_of_trehalose_in_the_preparation_of_specimens_for_molecular_electron_microscopy